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# Differences in Genetic Structure among *Fagus orientalis* Lipsky (Oriental Beech) Populations under Different Management Conditions: Implications for *in situ* Gene Conservation

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## Abstract

Resource sustainability requires a thorough understanding of the influence of forest management programs on the conservation of genetic diversity in tree populations. To observe how differences in forest management affect the genetic structure of *Fagus orientalis* Lipsky (oriental beech), we evaluated thirteen beech sites across Hyrcanian forests, based on six microsatellite loci. Significant differences between managed (mostly shelter wood system) and unmanaged populations was revealed. Inbreeding coefficient in managed populations was higher than unmanaged populations. A low, but significant, differentiation among all populations was found which reveals a clear geographic structure. Although the results indicate that the shelter wood system has minimum impact on the genetic diversity on a short term in oriental beech, but definitely inbreeding can increase in beech populations under intense management for long periods of time. According to these results, 4 populations from different part of Hyrcanian forests are suggested as potential *in situ* conservation sites.

**Keywords:** *Fagus orientalis* Lipsky; *In situ* conservation; Genetic diversity; Hyrcanian forests; Forest management

## Introduction

*Fagus orientalis* Lipsky (oriental beech) is a widespread monoecious and wind-pollinated tree species. The Iranian beech forests as part of the Hyrcanian forests are located on the northern slopes of Elborz Mountains[1], within an altitude of about 600-2000 m above sea level. They have formed a forest strip with

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[1] The watershed of the Elborz on the Caspian coast is characterized by mesophilous forest vegetation, originating from the Tertiary, therefore being very ancient. Beech in this zone survived intense climate and geological changes during the Quaternary because the populations lived in this area not reached by ice periods; only they have been influenced indirectly from glacial. From the point of view of its floristic composition, the beech belt is linked with European forests. This level particularly has some affinities with the beech forests of Balkans. The lower levels, on the contrary, are much more specific and include subtropical elements.

700 km length that is located in 3 provinces of Guilan, Mazandaran and Golestan. Pure and mixed beech (*F. orientalis*) forests are the most important elements of this ecosystem, making up the richest and the most beautiful forests of Iran. Beech forests represent about 18 % of Hyrcanian forests surface and form about 30 % of forest trees volume in Iran. Therefore, from the economic point of view, beech forests are the most valuable populations in Caspian zone and allocate the most rate of timber production in Iran. Hyrcanian commercial forests in Iran were nationalized in 1963. Since then, the area has declined significantly from 3.4 to less than 1.3 million ha in 1998. Beech forests in Iran are considered as even-aged forests and managed mostly by shelter wood silvicultural system.[1] Most of the beech dominated forests suffer from human interference and are continuously decreasing in area. Unsuitable harvesting methods during last 45 years and lack of forest protection are the two main technical reasons for failure of the shelterwood system in these forests [18].

A fundamental requirement for improved forest management is an understanding of the dynamics of genetic variation within and among tree populations. The gene pools of natural populations are not static, but constantly changing through the interactions of site characteristics (e.g., density, age structure, and dispersal distance) with the forces of evolution (mutation, mating system, gene flow, genetic drift, and natural selection). Forest management practices can alter these interactions and hence alter the partitioning of genetic variation within populations. Therefore, characterization of the changes in genetic diversity that result from forest management is essential for sustaining forest resources [28]. While easily measured phenotypes such as stem form, crown mass, and diameter growth are often used to evaluate the successful management of the quality of crop trees, effects at the genetic level have largely been ignored and are rarely incorporated into forest management programs [10].

Knowledge of genetic variation in the species is necessary if genetically well-adapted seed sources of Hyrcanian oriental beech are to be identified and used to establish populations. This type of information is also required to make sound decisions on how to protect the genetic diversity of the species and ensure sustainable forest management [20, 26, 31]. The conservation and protection of this tree species is most promising *in situ*, within the frame of forestry management, with the aim of combining gene conservation efforts and the production of high quality timber.

A new type of *in situ* gene conservation programs is developing the Gene Management Unit (GMU) concept, allowing evolutionary processes to take place in populations of plant and animal species while protecting genetic resources of important tree species as well as wild relatives of species. The selection of the GMU sites should be based on some knowledge of past and present human influence, mating conditions, and the size of minimum viable population; and of adaptively homogenous areas. Selected locations for GMU should adequately represent the spatial genetic and ecological variation pattern of the species, but the consideration of adaptive variation pattern should have priority. Knowledge of within-species genetic patterns arising from within-species gene flow and genetic adaptation is therefore essential for the decision on *in situ* gene conservation units [30].

Information on crucial life-history characteristics of forest tree species (e.g. mating system, pollen and seed dispersal) and their patterns of genetic variation is still lacking. In this context, the application of molecular genetic techniques to the conservation of forest tree species is anticipated to bring valuable data that could be used to extract relevant biological information, to document hotspots of genetic diversity, and to infer their phylogeography [36]. Salehi Shanjani et al. [39] used isozymes to study genetic diversity and differentiation beech populations in Hyrcanian forests. Like in European beech and many other trees species [3-7,13,15,16,22,27,29,32,33] high levels of diversity at isozyme loci and typically low population differentiation were observed. The lack of sufficient isozyme polymorphism among different populations limited their use. Molecular methods, at the DNA level, are now increasingly being used to study genetic diversity. One of the most reliable molecular marker systems is mircrosatellites, or simple sequence repeats (SSRs) which are abundant and well distributed throughout the nuclear genomes of eukaryotes. These are mostly present in non-coding DNA, which can accumulate mutations more rapidly than the coding DNA. SSRs can be used effectively to determine the magnitude and pattern of genetic variation in forest tree populations due to their high levels of polymorphism, high degree of reliability and reproducibility, and co-dominant mode of inheritance, and be a useful tool for quick decision making process in conservation of genetic resources *in situ* [45].

To reduce the cost and increase the efficiency of *in*

[1]Even-aged silvicultural system in which a new stand is established under the protection of a partial canopy of trees. The mature stand is generally removed in a series of two or more cuts, the last of which is when the new even-aged stand is well developed.

**Table 1.** Site characteristics of the studied beech populations

| Region | Population code | Altitude (m) | No. tree/Ha | Beech (%) | Canopy (%) | Manipulation level |
|---|---|---|---|---|---|---|
| Gorgan | G-1900 | 1900 | 182 | 96 | 95 | Unmanaged |
| | G-1400 | 1400 | 107 | 48 | 90 | H-managed* |
| | G-600 | 600 | 103 | 32 | 90 | H-managed* |
| Neka | N-1400 | 1400 | 60 | 60 | 80 | Unmanaged |
| | N-900 | 900 | 72 | 72 | 90 | H-managed* |
| Sangdeh | S-1900 | 1900 | 115 | 95 | 95 | Unmanaged |
| | S-1400 | 1400 | 52 | 71 | 70 | H-managed* |
| | S-900 | 900 | 82 | 67 | 65 | H-managed* |
| Kheirud-kenar | K-1200 | 1200 | 101 | 76 | 90 | L-managed** |
| | K-600 | 600 | 114 | 74 | 90 | L-managed |
| Clardasht | C-1300 | 1300 | 88 | 70 | 90 | Unmanaged |
| Asalem | A-1200 | 1200 | 180 | 42 | 90 | H-managed* |
| | A-600 | 600 | 119 | 37 | 70 | H-managed* |

*: High management density, under shelterwood system
**: limited management, by improvement or release cutting

**Table 2.** Characteristics of the 6 polymorphic nuclear microsatellite markers used to analyse genetic diversity in the Iranian beech populations

| Microsatellite locus | Repeat | Observed allele size range (bp) | Observed number of alleles | Annealing temp. (°C) |
|---|---|---|---|---|
| FS1-15 | $(GA)_{26}$ | 69-131 | 26 | 60 |
| FS1-03 | $(GA)_{18}$ | 68-120 | 18 | 60 |
| FS1-11 | $(GA)_{15}$ | 92-126 | 17 | 63 |
| FS3-04 | $(GCT)_5(GTT)_3(GCT)_6$ | 192-207 | 6 | 60 |
| FS4-46 | $(TGA)_{23}$ | 170-311 | 41 | 60 |
| FCM5 | $(AG)_{10}$ | 260-346 | 36 | 60 |

*situ* conservation sites in Hyrcanian forests, a few oriental beech populations covering a large part of the distribution range of *F. orientalis* in North of Iran were selected. This study aimed mainly to assess the general pattern of genetic variability of Hyrcanian beech forests at tree different geographical scales (among individuals within populations, among populations within natural regions, and between the regions). The implications of the results of this study for *in situ* conservation of genetic resources of Hyrcanian beech forests as Gene Management Units are also discussed. Additionally, the data were used for describing the impact of forest management on genetic diversity in oriental beech.

## Materials and Methods

Six regions covering a large part of the distribution range of *Fagus orientalis* were chosen in North of Iran: Asalem, Clardasht, Kheirud-Kenar, Sangdeh, Neka and Grogan (from the most west to the most east of beech distribution range) (Fig. 1). Each region consists of 1-3 sites in the lowest, middle and highest altitude of beech distribution range. The sites have a distinct difference in forest management history and intensity (Table 1). Dormant buds were sampled from adult trees (DBH > 30 cm). The sampled trees were randomly chosen and separated by at least 30 m.

DNA from bud material was extracted using a DNeasy Plant mini Kit (Qiagen). All trees were genotyped using six primer pairs of microsatellite loci (FS1-15, FS1-03, FS1-11, FS3-04, FS4-46 and FCM5) described in Pastorelli et al. [37]. All six SSR primers were approved to be of mendelian inheritance among crosses of *F. sylvatica* [37] and mapped to different linkage groups [41]. PCR process was performed following Pastorelli et al. [37]. PCR products were electrophoresed and visualized using an Amersham ALF Express automatic sequencer.

### *Data Analysis*

Data of all individuals were scored as a co-dominant marker and the following statistics of genetic variation within populations were computed as averages over loci using the GENAlEX 6 software [38]: mean number of alleles per locus (Na), effective number of alleles (Ne), number of rare alleles (Nr), the observed heterozygosity (Ho), expected heterozygosity (He) computed according to Nei's 1978 coefficient [35]. An estimator of Wright's Fst was calculated to assess population differentiation. Gene flow rate was estimated indirectly from the proportion of the total diversity found among populations (*Fst*, 47, 48). Wright's fixation index (*Fis*), averaged over all loci, was calculated and deviation from Hardy-Weinberg expectations were determined using GENEPOP 3.3. A paired t-test was performed to test for differences between the genetic diversity estimates in the populations with different management levels. Genetic distances were estimated according to Nei's 1978 coffeicient [35] and Neighbor-Joining (NJ) analysis were performed [14]. The NJ dendrogram was constructed with the MEGA 4 software [44]. To examine the relationship between the genetic distance and the geographic distance a Mantel test on the matrix of Fst values and that of the geographic distances was performed (1000 permutations) using GENEPOP 3.3. The results of the analysis were visualized by plotting the Fst values against the distances. A simulated annealing procedure implemented in the SAMOVA algorithm [8] was used to define groups of populations that are geographically homogeneous and maximally differentiated from each other. The program iteratively seeks the composition of a user-defined number K of groups of geographically adjacent populations that maximizes $F_{CT}$, the proportion of total genetic variance due to differences among groups of populations. The program was run for 10000 iterations for Ke {2, . . ., 8} from each of 500 random initial conditions. For each K, the configuration with the largest FCT values after the 500 independent simulated annealing processes was retained as the best grouping of populations.

## Results

### *Genetic Diversity*

A total of 144 alleles were detected ranging from 6 to 41 alleles, with an average of 24 alleles per locus (Table 2). The oriental beech forests in Hyrcanian zone are genetically diverse. All six microsatellite loci analyzed in this study were highly polymorphic, displaying a high number of alleles and a wide range of PCR products. Heterogeneity ($p<0.01$) in allele frequencies among the populations detected at all loci. Table 3 describes the polymorphism of studied samples. Most loci in all regions showed significant deviations from expected Hardy-Weinberg frequencies. In most populations there were more homozygous genotypes than expected. Two exceptional populations N-1400 and S-1900, both unmanaged, showed more heterozygosity than the amount expected (Table 3). Mean number of alleles per locus varied between 7.67 in G-1900 population to 11.67 in S-1900 and S-900 populations. Across all the loci examined 20 specific alleles (which occurred at low frequencies) were observed, distributed in 11 out of 13 populations. The overall allelic richness for Gorgan region was considerably lowest than others. Due to different manipulation levels, significant difference in average number of alleles per locus appeared within regions. There is no clear trend for differences in the diversity measures between the managed and unmanaged populations within a region, even between different altitudes (Table 3). Differences in heterozygosity within a region were considerable and significant. Observed heterozygosity was the highest (0.72) in N-1400 (unmanaged) and the lowest (0.48) in A-600 (managed under shelterwood system) populations. The expected heterozygosites ranged between 0.58 (in G-1400, managed population) and 0.72 (in C-1300, unmanaged, and K-1200, limited-managed populations). Mean heterozygoshty for all populations was 0.61 observed and 0.67 for expected. The observed heterozygosity was slightly lower than the expected heterozygosity (He), causing a low but significantly positive, mean inbreeding coefficient (Fis=0.061). The mean Fis was low, indicating that within population structure (inbreeding) was not significant. However some loci showed high values of Fis, indicating deficiency or excess of heterozygotes. Effective number of alleles, $n_e$, ranged between 3.29 (in G-1400, managed population) and 5.92 -5.43 (in C-1300 an unmanaged population, and K-1200 a limited-managed population, respectively). A pair-wise t-test for all regions did not

showed significant differences between the populations with different management levels.

### *Genetic Differentiation*

The overall Fst was low (0.058), but differed significantly from zero (P<0.001), meaning that 5.8% of total variation were among populations (Table 2). Also pair-wise comparisons of the populations within a region revealed very low Fst-values (P<0.001). The Asalem populations showed the lowest differentiation (Fst = 0.10) while the Gorgan populations were most divergent (Fst = 0.041) (Table 4).

For the description of the differentiation pattern, the genetic distances between populations were calculated using Nei´s unbiased estimator. The NJ phenogram was constructed using Nei's genetic distance (Fig. 2). The NJ tree had long terminal branches, which suggested that the populations were well differentiated, and that the relationships among the regions were well resolved. The branching order and the grouping of populations (clades) were consistent and supported the phenetic analysis. In clusteing analysis, two main branches were observed. The first main branch covered the regions Asalem, Clardasht and one population of Kheirud-kenar. The second main branch covered the regions Gorgan, Neka, Sangdeh and one population of Kheirud-Kenar. Gorgan-1900 population was the most genetically distant from all other populations and is significantly differentiated.

The genetic divergence between the 13 populations is a function of the geographic distance. A positive and significant correlation coefficient was calculated between the pair-wise Fst values and the geographic distances ($R^2 = 0.52$, P = 0.0003, Mantel test).

SAMOVA results also revealed a clear geographic pattern of the diversity among the analysed populations. Fct values increased progressively as K was increased, reaching a plateau (Fct = 10%) at k ≈ 4, with the Asalem and Clardasht and G-1900 populations significantly separated from the other populations (Fig. 3).

**Table 3.** Genetic variability at six microsatellite loci in 13 beech populations. sample size (N), mean number of alleles per locus (Na); effective number of alleles (Ne); observed (Ho) and expected (He) heterozygosities and fixation index (Fis)

| Population | N | Na | Ne | Nr | Ho | He | Fis |
|---|---|---|---|---|---|---|---|
| G-1900 | 35 | 7.67 | 3.47 | 1 | 0.55 | 0.62 | 0.063 |
| G-1400 | 64 | 8.50 | 3.29 | 0 | 0.57 | 0.58 | -0.035 |
| G-600 | 51 | 9.83 | 4.43 | 1 | 0.67 | 0.69 | -0.006 |
| *Overall* | | 8.67 | 3.73 | | 0.60 | 0.63 | 0.007 |
| N-1400 | 50 | 11.33 | 4.61 | 1 | 0.72 | 0.68 | -0.077 |
| N-900 | 49 | 10.50 | 4.36 | 1 | 0.61 | 0.67 | 0.043 |
| *Overall* | | 10.91 | 4.49 | | 0.67 | 0.68 | -0.017 |
| S-1900 | 37 | 11.67 | 4.65 | 1 | 0.67 | 0.64 | -0.028 |
| S-1400 | 44 | 10.83 | 4.67 | 2 | 0.59 | 0.66 | 0.059 |
| S-900 | 53 | 11.67 | 5.02 | 1 | 0.59 | 0.70 | 0.164 |
| *Overall* | | 11.39 | 4.78 | | 0.62 | 0.68 | 0.065 |
| K-1200 | 50 | 11.33 | 5.92 | 3 | 0.66 | 0.72 | 0.047 |
| K-600 | 39 | 10.33 | 3.57 | 4 | 0.58 | 0.66 | 0.113 |
| *Overall* | | 10.83 | 4.75 | | 0.62 | 0.69 | 0.08 |
| C-1300 | 23 | 11.00 | 5.43 | 3 | 0.57 | 0.72 | 0.225 |
| A-1200 | 50 | 11.33 | 4.75 | 2 | 0.59 | 0.65 | 0.028 |
| A-600 | 40 | 9.00 | 4.26 | 0 | 0.48 | 0.65 | 0.204 |
| *Overall* | | 10.17 | 4.51 | | 0.54 | 0.65 | 0.116 |
| *Overall* | | 10.42 | 4.49 | | 0.61 | 0.67 | 0.056 |

**Table 4.** F-statistics and gene flow values among beech populations using six microsatelites

| Reigon | N | Fis | Fit | Fst | Nm |
|---|---|---|---|---|---|
| 1) Among all populations | 13 | 0.060 | 0.115 | 0.058 | 4.060 |
| 2) Pair-wise per region | | | | | |
| Gorgan | 3 | 0.006 | 0.048 | 0.041 | 5.840 |
| Neka | 2 | -0.016 | -0.001 | 0.015 | 16.540 |
| Sangdeh | 3 | 0.062 | 0.080 | 0.018 | 13.522 |
| Kheirud-kenar | 2 | 0.082 | 0.103 | 0.025 | 9.764 |
| Asalem | 2 | 0.117 | 0.124 | 0.010 | 24.468 |

## Discussion

### *Inbreeding*

Observed heterozygosity values were lower than the amounts expected heterozygosity in eleven of the thirteen sampled populations. In the other word, most oriental beech populations showed a tendency towards a heterozygote deficit, which is in concordance with results reported using isozymes in same populations of Hyrcanian forests (average Fis = 0.047, 39). Buiteveld et al. [2] in European beech using microsatelites also fond positive values of the inbreeding coefficient (average Fis = 0.224), indicating significant heterozygote deficiencies. Other studies in beech using isozymes generally also showed a tendency towards a heterozygote deficit (average Fis = 0.065, 5; Fis = 0.115, 6; average Fis = 0.117, 27; Fis = 0.037–0.041,

24). Also other wind-pollinated, broad-leaved tree species, including other *Fagus* species showed heterozygote deficiencies such as *F. crenata* (average Fis = 0.095, 1), *Fraxinus* excelsior (significant Fis = 0.014; 17, Fis = 0.292; 34), oak (Fis = 0.07; 42). Most authors explain positive inbreeding coefficients in beech as a result of a non-random microspatial structure probably due to mating in close neighborhood [5, 7, 32, 46]. Also more recently, Vornam et al. [45], who studied the spatial distribution of genetic diversity in a naturally regenerated beech population with microsatellites detected high Fis values and found a strong family structure up to 30 m.

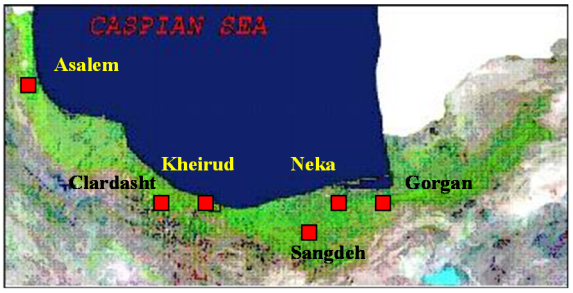


**Figure 1.** Distribution of studied regions.

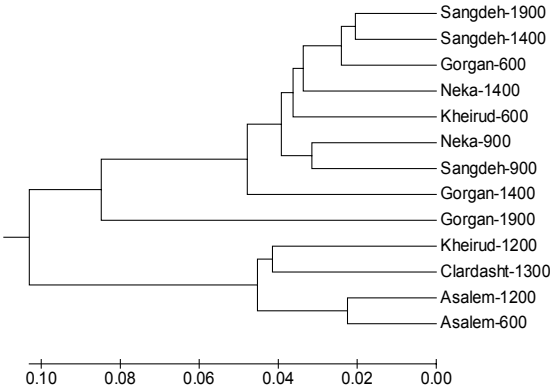


**Figure 2.** Dendrogram of different populations produced by the neighbour-joining clustering method.

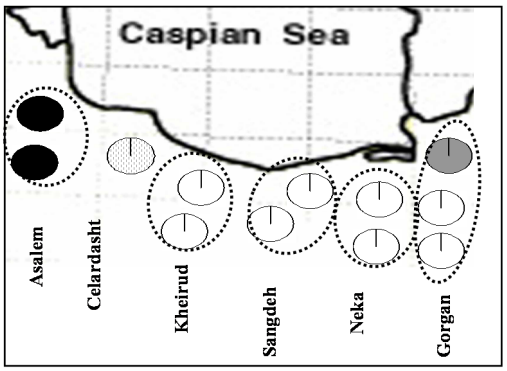


**Figure 3.** Group structure of the 13 beech populations defined by SAMOVA (samples with the same patterns belong to the same group).

In most cases, Fis values were highest in intensively managed populations within a region, suggesting that human interference or management increase inbreeding levels. This is because, for wind-pollinated species, a low density population of mature trees is expected to increase the spatial genetic structure of the population by increasing the probability of pollination by spatially proximal relatives [11]. Likewise, inbreeding should be limited in a high density forest because the pollen cloud for a tree in a dense population would contain a relatively small proportion of its own pollen; therefore, most seeds would be a product of outcrossing [9, 12, 21]. Clearly, inbreeding can increase in oriental beech populations under intense management for long periods of time.

### *Genetic Differentiation between Beech Populations*

Microsatellites showed that most of the genetic diversity was attributed within the populations and that there was little, but significant differentiation between the populations (Fst = 0.058). This pattern of variation is thought to be the consequence of the dominant life history characteristics of forest trees, i.e. long life span, allogamy, wind-pollination, their continuous distribution and monoecy, and also may be with a high rate of gene flow (15). This low differentiation is in concordance with Fst values reported with isozymes in Hyrcanian beech forests (Fst = 0.034, 39), and with SSRs (Fst = 0.058, 2) and isozymes (Fst = 0.03–0.07, 3), which have been performed with many European beech populations over a wide range of the species distribution. Moreover, despite the low genetic differentiation among the populations, a clear geographical pattern was observed.

Cluster analysis (dendrogram) based on Nei's 1978 genetic distance also showed that all populations were separated according to their location in eastern or western part of Hyrcanian forests. Considerable amount of gene flow between the populations of each cluster probably occurs considering the topography of the area, prevailing wind direction and physical distance between these populations. In the first cluster the G-1900 population was the most distantly clustered one compared to other populations of eastern part. These

findings are not surprising since G-1900 population occurs in extreme habitats (e.g., high elevation, shallow soil), at the eastern edge of the species range in Hyrcanian forests. On the other hand, S-900 and N-900, which are in the same cluster, suffered from sever management practices. The only exception is K-600, which we expected to group in the second cluster close to K-1200. In second cluster, K-1200 was clearly separated from the other three populations. A-600 and A-1200 were the most closely related ones.

The SAMOVA analysis showed that the eastern populations are more similar to each other and are distinct from the central and western populations. This division is most obviously a result from historical migration of the species in Hyrcainan forests, which is from West to East [40].

### *Effect of Forest Management on Beech Populations*

In this study, the potential effects of forest management on beech populations were examined. Natural beech populations were used as a reference for comparison of genetic diversity in populations which undergone a forest management treatment. The pair-wise comparison revealed no differences in genetic diversity measures among the populations with different management history and therefore it can be concluded that the management practiced in these populations did not influence the level of genetic diversity at the genetic markers used. Other studies in European beech aimed at studying the effect of management on the genetic diversity did not find also an effect ([2], suggesting the shelterwood system has no or minimum impact on genetic diversity in European beech. Furthermore, they observed Linkage disequilibrium for some loci pairs in their study, but no more in the managed populations than in the limited populations within a region. Hussendörfer and Konnert [19] compared isozyme data of 112 managed and 13 natural, unmanaged beech forests located in South Germany, but did not find significant differences in genetic diversity parameters. Two other studies focused on the effect of forest management, such as thinning [25] and diameter oriented selection felling [22] on the diversity: also in these cases no biological significant differences were detected. On the other hand, a study in a related beech species (*F. crenata* Blume) showed that forest cutting decreased the genetic variability and increased the genetic clustering in the population [43]. Furthermore, they observed significant linkage disequilibrium in the population with a cutting history, but none in the primary population. The effects of forest cutting were explained by the limited number of reproductive trees that were left after cutting and consequently reduced mixing of the progenies.

Finally, it can be concluded from our results that the shelter wood system is adequate to avoid changes of the genetic composition on a short-term in oriental beech. It is likely that this type of management has minimum impact on the diversity in beech, at least for the genetic markers used, because the management mimics natural regeneration processes. However our results clearly showed that inbreeding can increase in oriental beech populations under intense management for long periods of time. Only with an increased understanding of the changes that occur as a result of forest management will it be possible to conserve genetic diversity in tree populations - a crucial step for sustainable resource management.

### *Geographic Diversity and* **in Situ** *Gene Conservation*

Protection of all studied beech populations in the Hyrcainan forests may not be economically feasible. The number and size of Gene Management Unit (GMU) as an *in situ* reserve could not be determined by the genetic data presented here alone since there will be other biological, economical and administrative issues which should be considered during decision making. However based on present genetic data, we could point out some potential populations as GMU sites along with distribution range of beech forests to help forest managers and conservation biologists in their decision making.

We applied SAMOVA for our data, which defined 4 groups of populations in Hyrcanian zone that are geographically homogeneous and maximally differentiated from each other (Fig. 3). The SAMOVA approach also suggests the different groups in very close populations, e.g. populations from different elevation of region Gorgan or two populations in two sides of a valley (populations Kelardasht and Kheirud-kenar). To explain it, the presence of a very efficient barrier to gene flow, this would have prevented short-range migrations between populations from these regions.

In Beech forests, considering the genetic distances between populations and grouping pattern and genetic diversity statistics of populations (heterozygosity and mean number of alleles per locus), G-1900, K-1200, C-1300 and A-1200 could be suggested as potential GMZ populations. These populations are the representatives of the cluster groups formed in cluster analysis. Asalem and Gorgan regions have located at the western and eastern edge of Hyrcanian forests, respectively. Gene flow from the other populations is low. Therefore, It is reasonable that G-1900 and A-1200 (has rare alleles and

higher heterozygosity) originated from different lineages can be considered as *in situ* reserves. K-1200 and C-1300 populations in middle of distribution range of beech in Hyrcanian forests have rare alleles and the highest heterozygosity and originated from different lineages could be taken into consideration for *in situ* conservation.

However, for the final decision on Gene Management Unit selection in beech forests as *in situ* reserve, genetic diversity of a species is not the unique criterion. Other factors such as presence of other economically important species and degree of biological diversity in the site, accessibility and suitability of the area for *in situ* preservation and protection should be also taken into consideration.

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## References

1. Asuka Y., Tomaru N., Nisimura N., Tsumura U., and Yamamoto S. Heterogeneous genetic structure in a *Fagus crenata* population in an old-growth beech forest revealed by microsatellite markers. *Mol. Ecol*., **13**: 1241–1250 (2004).
2. Buiteveld J., Vendramin G.G., Leonardi S., Kamer K., and Geburek, T. Genetic diversity and differentiation in European beech (*Fagus sylvatica* L.) stands varying in management history. *For. Ecol. Manag.*, **247**: 98-106 (2007).
3. Comps B., Gömöry D., Letouzey J., Thie´baut B., and Petit R.J. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. *Genetics*, **157**: 389–397 (2001).
4. Comps B., Ma´tya´s C., Letouzey J., and Geburek T. Genetic variation in beech populations (Fagus sylvatica L.) along the alpine chain and in the Hungarian basin. *For. Genet*., **5** (1): 1–9 (1998).
5. Comps B., Thie´baut B., Paule L., Merzeau D., and Letouzey J. Allozymic variability in beech woods (*Fagus sylvatica* L.) over Central Europe: spatial differentiation among and within populations. *Heredity*, **65**: 407–417 (1990).
6. Comps B., Thiebaut B., Sugar I., Trinajstic I., and Plazibat M. Genetic variation of the Croatian beech stands (*Fagus sylvatica* L.): spatial differentiation in connection with the environment. *Ann. For. Sci.*, **48**: 15–28 (1991).
7. Cuguen J., Merzeau D., and Thiebaut B. Genetic structure of the European beech stands (*Fagus sylvatica* L.): F-statistics and importance of the mating system characteristics in their evolution. *Heredity*, **60**: 91–100 (1988).
8. Dupanloup I., Schneider S., and Excoffier L. A simulated annealing approach to define genetic structure of populations. *Mol. Ecol.,* **11**: 2571– 2581 (2002).
9. El-Kassaby Y.A., Dunsworth B.G., and Krakowski J. Genetic evaluation of alternative silvicultural systems in coastal montane forests: western hemlock and amabilis fir. *Theor. Appl. Genet.,* **107**: 598–610 (2003).
10. Ennos R.A., Worrell R., and Malcolm D.C., The genetic management of native species in Scotland. *Forestry*, **71**: 1-23 (1998).
11. Epperson B.K. *Geographical Genetics.* Princeton University Press, Princeton, N.J., 230 p. (2003).
12. Farris M.A., and Mitton J.B. Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. *Evolution*, **38**: 1151-1154 (1984).
13. Gömöry D., Vyšny` J., Comps B., and Thiebaut B. Geographical patterns of genetic differentiation and diversity in European beech (*Fagus sylvatica* L.) populations in France. *Biológia (Bratislava)*, **47**: 571–579 (1992).
14. Gower J.C. Some distance properties of latent root and wector methods used in multivariate analysis. *Biometrika*, **53**: 325-338 (1966).
15. Hamrick J.L., Godt M.J.W., and Sherman-Broyles S.L. Factors influencing levels of genetic diversity in woody plant species. *New Forestry*, **6**, 95–124 (1992).
16. Hazler K., Comps B., Sugar I., Melovski L.J., Tashev A., and Gračan J. Genetic structure of *Fagus sylvatica* L. populations in Southeastern Europe. *Silvae Genetica*, **46**(4): 229–236 (1997).
17. Heuertz M., Hausman J.-F., Tsvetkov I., Frascaria-Lacoste N., and Vekemans X. Assessment of genetic structure within and among Bulgarian populations of the common ash (Fraxinus excelsior L.). *Mol. Ecol.*, **10**: 1615–1623 (2001).
18. Hosseini S., Madjnonian B., and Nieuwenhuis M. Damage to natural regeneration in the Hyrcanian forests in Iran: a comparison of two typical timber extraction operations. *J. For. Eng*., **11**: 69-73 (2000).
19. Hussendörfer E., and Konnert M. Untersuchungen zur Bewirtschaftung von Weisstannen- und Buchenbeständen unter dem Aspekt der Erhaltung genetischer Variation. *For. Snow Landsc. Res.*, **75**(1.2): 187–204 (2000).
20. Kaya Z., Kün E., and Güner A. National plan for *in situ* conservation of plant genetic diversity in Turky. Milli Eğitim Basimevi, İstanbul, 123 p. (1997).
21. Knowles P., Fumier G.R., Aleksiuk M.A., and Perry D.J. Significant levels of self-fertilization in natural populations of tamarack. *Canadian J. Bot.*, **65**: 1087-1091 (1987).
22. Konnert M., and Spiecker H. Beeinflussen Nutzungen einzelner Bäume die genetische Struktur von Beständen? *AFZ-Der Wald*, 51(23): 1284–1291 (1996).
23. Konnert M., Ziehe M., Tröber U., Maurer W., Janssen A., Sander T., Hussendörfer E., and Hertel H. Genetic variation of beech (*Fagus sylvatica* L.) in Germany: joint evaluation of genetic inventories from several federal states. *For. Holz*., **13**: 403–408 (2000).
24. Larsen, A.B. Genetic structure of populations of beech (*Fagus sylvatica* L.) in Denmark. *Scandinavian J. For.*

*Res.*, **11**: 220–232 (1996).
25. Lauber von U., Rotach, P., and Hussendörfer, E. Auswirkungen waldbaulicher Eingriffe auf die genetische Structur eines Buchen-Jungbestandes (*Fagus sylvatica* L.). *Schweiz Forstwes*., **148**(11): 847–862 (1997).
26. Ledig T.F. Genetic variation in Pinus. In: Richardson D.M. (Ed.), *Ecology and biogeography of Pinus*. Cambridge University Press, New York, pp. 251-280 (1998).
27. Leonardi S., and Menozzi P. Genetic variability of *Fagus sylvatica* L. in Italy: the role of postglacial recolonisation. *Heredity*, **75**: 35–44. (1995).
28. Lindenmayer D., and Franklin J. Transitions to ecological sustainability in forests - a synthesis. In: Lindenmayer D.B., and Franklin J.F. (Eds.), *Towards forest sustainability*. Island Press, Washington, D.C., pp. 205-213 (2003).
29. Magri D., Vendramin G.G., Comps B., Dupanloup I., Geburek T., Gömöry D., Latalowa M., Litt T., Paule L., Roure J.M., Tantau I., van der Knaap W.O., Petit R.J., and de Beaulieu J.-L. A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist*, **171**: 199–221 (2006).
30. Mátyás C.S. Population, conservation and ecological genetics. In**:** Burley J., Evans J., Youngquist J. (Eds.), *Encyclopedia of Forest Sciences*. Elsevier Major Reference Works, Oxford., 240 p. (2004).
31. Meffe G.K., and Carroll C.R. Principels of conservation Biology. Sinauer Associates, Inc. Sunderland, Massachusetts, 600 pp. (1994).
32. Merzeau D., Comps B., Thiebaut B., Cuguen J., and Letouzey J. Genetic structure of natural stands of *Fagus sylvatica* L. (beech). *Heredity*, **72**: 269–277 (1994a).
33. Merzeau D., Comps B., Thiebaut B., and Letouzey J. Estimation of *Fagus sylvatica* L. mating system parameters in natural populations. Ann. For. Sci., **51**: 163–173 (1994b).
34. Morand M.-E., Brachet S., Rossignol P., Dufour J., and Frascaria-Lacoste N. A generalized heterozygote deficiency assessed with microsatellites in French common ash populations. *Mol. Ecol.,* **11**: 377–385 (2002).
35. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583-590 (1978).
36. Newton A.C., Allnutt T.R., and Gillies A.M. Molecular phylogeography, intraspecific variation and the conservation of tree species. *Trends Ecol. Evol*., **14**: 140-145 (1999).
37. Pastorelli R., Smulders M.J.M., Van'T Westender W.P.C., Vosman B., Giannini R., Vettori C., and Vendramin G.G. Characterization of microsatellite markers in *Fagua sylvatica* L. and *Fagus orientalis* Lipsky. *Mol. Ecol. Notes,* **3**: 76-78 (2003).
38. Peakal R., and Smouse P.E. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes,* **6**: 288–295 (2006).
39. Salehi Shanjani P., Paule L., Khavari-Nejad R.A., Gömöry D., Sagheb-Talebi K. Allozymic variability in beech (*Fagus orientalis* Lipsky) forests over Hyrcanian zone. *For. Genet.,* **9**(4): 297-297 (2002).
40. Salehi Shanjani P., Vettori C., Giannini R., and Khavari-Nejad R.A. Intraspecific variation and geographic patterns of Fagus orientalis Lipsky chloroplast DNA. J. *Silveae Genetica,* **53** 193-197 (2004).
41. Scalfi, M., Troggio, M., Piovani, P., Leonardi, S., Magnaschi, G., Vendramin, Menozzi, P. 2004. A RAPD, AFLP and SSR linkage map, and QTL analysis in European beech (*Fagus sylvatica* L.). Theoretical Applied Genetics 108, 433–441.
42. Streiff R., Labbe T., Bacilieri R., Steinkellner H., Glössl J., Kremer A. Within-population genetic structure in *Quercus robur* L. and Quercus petraea (Mattl) Liebl. assessed with isozymes and microsatellites. *Mol. Ecol.,* **7**: 317–328. (1998).
43. Takahashi M., Mukouda M., and Koono K. Differences in genetic structure between two Japanese beech (*Fagus crenata* Blume) stands. *Heredity*, **84**: 103–115 (2000).
44. Tamura K., Dudley J., Nei M., Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, 24; 1596-1599 (2007).
45. Vendramin G.G., Scotti I., Ziegenhagen B. Microsatellites in forest trees: characteristics, identification and applications. In: Kumar S., and Fladung M. (Eds.), *Molecular Genetics and Breeding of Forest Trees*. The Haworth Press, Inc. Bringhamton, NY, USA, pp. 359-386 (2004).
46. Vornam B., Decarli N., Gailing O. Spatial distribution of genetic variation in a natural beech stand (*Fagus sylvatica* L.) based on microsatellite markers. *Conserv. Genet*., **5**: 561-570 (2004).
47. Wright S. Evolution in mendelian populations. *Genetics*, **16**: 97-159 (1931).
48. Wright S. The genetic structure of populations. *Ann. Eugen.*, **15**: 323-354 (1951).